

The RANTES promoter polymorphism: A genetic risk factor for near-fatal asthma in Chinese children

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Background: RANTES promoter polymorphisms were found associated with asthma/atopy in some studies but not others, possibly reflecting the genetic heterogeneity among different ethnicities and different asthma severity.

Objective: The purpose of this investigation was to test the genetic association between the RANTES –28C/G and –403G/A polymorphisms and asthma/atopy in a cohort of Chinese children, with particular emphasis on those patients who had experienced life-threatening asthma attacks.

Methods: Forty-eight children with near-fatal asthma, 134 children with mild-to-moderate asthma, 69 children with allergic disorders but no asthma, and 107 nonasthmatic nonatopic control children were genotyped through use of a PCR-based assay.

Results: No significant difference was demonstrated for frequency of the RANTES –28C/G polymorphism when the mild-to-moderate asthma, atopic/nonasthmatic, and normal control groups were compared. The RANTES –28G allele was present in a significantly higher proportion of the children with near-fatal asthma compared with the nonasthmatic nonatopic controls (odds ratio, 2.93 [1.41–6.06]; $P = .006$) and the children with mild-to-moderate asthma (odds ratio, 3.52 [1.73–7.16]; $P = .001$). The frequency of –28G allele carriage correlated with asthma severity. The RANTES –28G allele was also associated with an increased blood eosinophil count and a higher degree of bronchial hyperresponsiveness. The RANTES –403G/A polymorphism did not influence asthma/atopy susceptibility, blood eosinophil count, or bronchial hyperresponsiveness.

Interestingly, a higher frequency of –403A allele carriage was observed in the moderate asthma subgroup compared with the mild asthma analog.

Conclusions: We conclude that the RANTES –28C/G polymorphism exacerbates asthma severity, representing a genetic risk factor for life-threatening asthma attacks in Chinese children. In addition, the linkage disequilibrium between these 2 poly-

morphisms is a potential confounder that must be considered in the design and interpretation of RANTES gene association studies. (*J Allergy Clin Immunol* 2003;111:1285–92.)

Key words: Asthma, near-fatal asthma, polymorphism, RANTES, chemokine, Chinese, children

The prevalence and severity of asthma have been increasing in many countries, the trends being most pronounced for children and adolescents.¹ For example, the prevalence of childhood asthma in Taiwan has increased dramatically during the last 2 to 3 decades. A series of surveys has been conducted by a number of our colleagues in Taiwan over a 20-year period (1974–1994), with current asthma prevalence assessed for children of the same age at the same schools through use of the same method.^{2,3} This prevalence has increased from 1.3% in 1974 to 10.8% in 1994. Our recent work has also revealed that the severity of asthma seems to have increased, as reflected by a significant upward trend in admission rates for cases of childhood asthma.³ Thus, childhood asthma is today a major health problem not only in the Western countries but in Taiwan as well.

Asthma is a disease with a strong genetic predisposition, and the recent increase in asthma cases seems to be triggered by increases in environmental exposures in previously unaffected but genetically susceptible individuals.⁴ Genetic susceptibility is probably caused by a characteristic pattern of polymorphisms in the multiple genes involved in the regulation of immunologic responses.⁴ It is therefore important to identify these polymorphisms in candidate genes. Through use of genome-wide searches, several candidate genes for asthma and atopy have been described^{4–6}; one of these is named *RANTES* (for “regulated upon activation normal T cell expressed and secreted”). The *RANTES* gene lies on chromosome 17q11.2–q12,⁷ a region for which linkage to asthma or atopy has been demonstrated in several independent studies.^{4–6} *RANTES* can produce chemotaxis and activation of several cell types (eg, eosinophils, monocytes, basophils, and T cells—particularly CD4⁺CD45RO⁺ memory T cells) that are central to the airway inflammation characteristic of asthma.⁸ These results imply a pivotal role for *RANTES* in the pathogenesis of asthma. This hypothesis is strengthened by the fact that the *in vivo* neutralization

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Abbreviations used

BHR: Bronchial hyperresponsiveness

RFLP: Restriction fragment length polymorphism

of RANTES significantly reduces the allergic airway inflammation in a murine model.⁹ In addition, increased RANTES mRNA expression has also been demonstrated in bronchial biopsy specimens derived from patients with atopic asthma and patients with nonatopic asthma,¹⁰ and higher RANTES levels have been demonstrated in the bronchoalveolar lavage fluid of patients with active asthma.¹¹ Furthermore, when the same asthmatic patients were compared, significant elevation of plasma RANTES level has been demonstrated during acute attacks compared with the asymptomatic state.¹² In addition, a large body of evidence suggests that the eosinophil is a key proinflammatory leukocyte in allergic asthma, the number recruited to the lung being strongly associated with immunoreactive RANTES concentration.¹³ All of these results indicate that RANTES is an attractive gene candidate for the genetic dissection of asthma.

It has been demonstrated that 2 functional polymorphisms in the proximal promoter region of the RANTES gene (–28 C to G and –403 G to A) increase transcriptional activity and subsequent RANTES expression in human cell lines.^{14,15} It is therefore quite likely that these polymorphisms are possible loci involved in asthma susceptibility or modulation of its severity. Nickel et al¹⁵ have shown that the –403A allele is associated with atopic dermatitis but not with asthma in German children. Furthermore, it has been demonstrated for a Caucasian population that this mutant allele is associated with increased susceptibility to both asthma and atopy and increased severity of airway obstruction.¹⁶ However, the association between the RANTES promoter polymorphisms (either –28C/G or –403G/A) and asthma/atopy was not confirmed for sample populations of Hungarian children.^{17,18} These inconsistent results might be a reflection of genetic heterogeneity intrinsic to different ethnicities, reflecting variability in the relative importance of risk alleles for different gene pools. Asian populations have never been included in previous studies of the association between the aforementioned polymorphisms and asthma and/or allergic disorders. It has been suggested, however, that there are significant interethnic differences in the allele frequencies for asthma predisposition genotypes between Asian and Caucasian populations.¹⁹ With regard to the RANTES promoter polymorphisms, McDermott et al²⁰ observed such ethnic variation when they compared different ethnic populations. Furthermore, the respective prevalence of these 2 mutant alleles was estimated to be ~17% (–28G) and 37.6%–39.3% (–403A) in the Japanese population^{14,21}; these are strikingly higher than analogous figures reported for Western samples.^{15–18,20,22,23} Thus, it is important to analyze the impact of the RANTES promoter polymorphisms on asthma or atopy for populations living in Asia, because these polymorphisms are possibly endemic to the

region. Furthermore, there is evidence for a genetic contribution to risk for fatal or near-fatal asthma attacks.²⁴ In addition, recent work has demonstrated that the levels of RANTES, monocyte chemoattractant protein 1, and macrophage inflammatory peptide 1 α are increased in the bronchoalveolar lavage fluid of ventilated subjects with status asthmaticus compared with patients with mild-to-moderate asthma, suggesting that CC chemokines might be important factors in life-threatening asthma attacks.²⁵

In this study, we hypothesized that RANTES promoter polymorphisms that influence the expression of this CC chemokine are risk factors for life-threatening asthma attacks and/or susceptibility to asthma or atopy. To test this hypothesis, we investigated the frequency of the RANTES –28C/G and –403G/A polymorphisms for a cohort of Chinese children, with particular emphasis on those patients who had experienced life-threatening asthma attacks.

MATERIALS AND METHODS

Study subjects

This study was conducted at Chang Gung Children's Hospital, a tertiary teaching medical center in northern Taiwan. A total of 358 unrelated Chinese children (aged 7.4 ± 3.5 years) were enrolled and divided into 4 groups, as follows: (1) those who had experienced near-fatal asthma attacks ($n = 48$; aged 5.9 ± 4.0 years); (2) those who have mild-to-moderate asthma ($n = 134$; aged 7.8 ± 2.8 years); (3) those who have allergic disorders but no asthma ($n = 69$; aged 8.1 ± 3.9 years); and (4) normal controls ($n = 107$; aged 7.3 ± 3.6 years).

The first group consisted of subjects who either had histories of hospital admission requiring intubation and ventilation for acute exacerbation of asthma symptoms or had experienced hypercapnic respiratory failure during an acute asthmatic episode with a PaCO₂ of >45 mm Hg. At the time of this writing, there have been no asthma fatalities at our hospital involving individuals aged 16 years or less during the previous 10 years. Thus, no asthma fatalities were included in this cohort. The second group consisted of subjects with mild-to-moderate asthma who had never experienced fatal or near-fatal asthma episodes and were not taking oral steroids. The diagnosis and classification of the clinical severity of asthma was made according to the GINA guidelines.²⁶ These patients with mild-to-moderate asthma were further stratified into 2 subgroups according to the clinical features before treatment and lung function over 12 months: those with mild asthma and those with moderate asthma. The inclusion criteria for the mild asthma subgroup included a symptom frequency of less than once a day, nocturnal symptoms less than once a week, brief exacerbations, no symptoms and normal peak expiratory flow between exacerbations, a percent predicted FEV₁ of $\geq 80\%$, and an FEV₁ variability of $\leq 30\%$. The inclusion criteria for the moderate asthma subgroup included daily or continual symptoms, frequent exacerbations affecting activity and sleep, frequent nocturnal symptoms, a percent predicted FEV₁ of $<80\%$, and an FEV₁ variability of $>30\%$. This stratification of patients with mild-to-moderate asthma was performed without prior knowledge of the subjects' genotypes. The third group consisted of atopic nonasthmatic subjects who had specialist-physician allergy diagnoses according to the following criteria: (1) atopy; (2) clinical signs of severe allergy (including allergic rhinitis, atopic dermatitis, food allergy, and urticaria); and (3) no symptoms or history of asthma. The fourth group consisted of age-comparable normal controls who were selected from children attending the hospital for nonallergic and nonimmunologic diseases who met the following criteria:

(1) no symptoms or history of allergic diseases; and (2) total serum IgE levels below the general population mean for their ages. Both of the parents of each enrolled subject had to be ethnic Chinese. Informed consent was obtained from the parents or guardians of each subject before commencement, and the study was preapproved by the hospital ethics committee.

Genotyping

Genomic DNA was extracted from whole blood through use of an Easy Pure Genomic DNA Purification Kit (Bioman Scientific Co, Taipei, Taiwan) for all study subjects. A PCR restriction fragment length polymorphism (RFLP) method, as described by Szalai et al,²⁷ was used to genotype patients and controls for the target polymorphisms. The RANTES –28 genotype was determined through use of a *HincII* site introduced with a mismatch into the PCR primer next to the C/G transition. Amplification with the primers RANTES-50S: 5'-ACT CCC CTT AGG GGA TGC CCG T-3', which has a guanine instead of a cytosine (underlined), and RANTES124AS: 5'-GCG CAG AGG GCA GTA GCA AT-3' generated a 175-bp product. Digestion with *HincII* yields 152- and 23-bp fragments when C is at position –28. The RANTES –403 genotype was determined through use of a *RsaI* site introduced with a mismatch into the PCR primer next to the G/A transition. Amplification with the primers RANTES-581S: 5'-CAC AAG AGG ACT CAT TCC AAC TCA-3' and RANTES-376AS: 5'-GTT CCT GCT TAT TCA TTA CAG ATC GTA-3', which has a guanine instead of a thymine (underlined), generated a 206-bp product. Digestion with *RsaI* yields 180- and 26-bp fragments when G is at position –403. Genomic DNA (100 ng) was amplified in a 25- μ L PCR reaction under the following cycling conditions: denaturation at 94°C for 5 minutes, followed by 35 cycles at 94°C for 1 minute, 55°C for 1 minute, and 72°C for 55 seconds, with a final extension at 72°C for 10 minutes. Five microliters of the PCR products were digested with 5 U of *HincII* or *RsaI* (Roche Diagnostics, Mannheim, Germany) at 37°C for 3 hours. The digestion products were analyzed on a 3% agarose gel stained with ethidium bromide. Both the direct sequencing reactions, through use of the ABI prism 377 automated DNA sequencer (Applied Biosystems, Foster City, Calif), and restriction enzyme digestion, resulted in identical genotypes for the initial 15 samples, all other samples being subsequently genotyped by PCR-RFLP.

Total and allergen-specific IgE, blood eosinophil counts, and bronchial hyperresponsiveness

Peripheral venous blood was collected from patients and control subjects for measurement of serum total and specific IgE levels in response to common aeroallergens through use of fluorescent enzyme immunoassay (AutoCAP System; Pharmacia Diagnostics AB, Uppsala, Sweden). Total serum IgE levels were defined as high when they exceeded the general population mean for their respective ages; specific IgE was considered positive in those having detectable allergen-specific IgE (≥ 0.35 kU/L). Children were defined as atopic if they had at least 1 positive result for allergen-specific IgE to aeroallergens common to Taiwan, including *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, cockroach, cat and dog dander, and *Candida albicans*. The absolute peripheral blood eosinophil counts were measured through use of a Sysmex SE-9000 Automated Hematology Analyzer (TOA Medical Electronics Co, Kobe, Japan). Bronchial challenge tests were performed as described previously.²⁸

Statistical analysis

Data analysis was performed through use of the SPSS statistical package (SPSS 10.0 for Windows, SPSS Inc, Chicago, Ill). The

genotype frequencies for the different groups were compared through use of the Pearson χ^2 test. Odds ratios (ORs) with 95% CIs were calculated as measures of the association between each genotype and asthma. The genotype distributions for the 2 polymorphisms tested in our study population were found to be in Hardy-Weinberg equilibrium. Haplotype frequencies for the 4 study groups were estimated through use of the EH program.²⁹ Blood eosinophil counts, total serum IgE levels, and PC₂₀ values between different groups were compared by means of the Mann-Whitney *U* test or the Kruskal-Wallis test, as appropriate. *P* values of $<.05$ were considered statistically significant.

RESULTS

Association of RANTES genotypes with near-fatal asthma, mild-to-moderate asthma, and atopy

No significant difference was found among the 3 asthmatic or atopic groups for total serum IgE levels. The RANTES –28 genotype and allele frequencies for the 4 study groups are presented in Table I. No significant difference was demonstrated for frequency of the RANTES –28C/G polymorphism or genotype distribution when the mild-to-moderate asthma, atopic-nonasthmatic, and normal-control groups were compared. We were unable to show an increased prevalence for this polymorphism in children with mild-to-moderate asthma compared with normal controls. Furthermore, this result was not significantly changed if the near-fatal and mild-to-moderate asthma groups were combined and compared with normal controls. The RANTES –28G allele was present (in either the heterozygous or the homozygous state) in a significantly higher proportion of the patients with near-fatal asthma compared with the nonasthmatic-nonatopic controls (OR, 2.93; *P* = .006) and their mild-to-moderate counterparts (OR, 3.52; *P* = .001; Table I). Associations of similar significance were demonstrated when allele frequencies for the 4 groups were compared.

There were no significant between-group differences demonstrated with respect to RANTES –403G/A-polymorphism frequency or genotype distribution (Table I). Table II shows the estimated haplotype frequencies for all study groups. The haplotype distributions were similar between the mild-to-moderate asthma, atopic nonasthmatic, and normal control groups. The frequency of haplotype III (–28G, –403G) was markedly higher for the near-fatal asthma group compared with the aforementioned 3 groups (*P* < .001 in all cases). This was also the case for the haplotype IV (–28G, –403A), though statistical significance was not achieved.

Association of RANTES genotypes with asthma severity

The subjects with mild-to-moderate asthma were further stratified into 2 subgroups—those with moderate asthma and those with mild asthma—and then separated according to their genotypes at the RANTES –28 or –403 loci, respectively (Table III). The frequencies for carriers of the –28G allele in the moderate asthma and

TABLE I. Genotype and allele frequencies for 4 groups of Chinese children

Group	n	Genotype				Allele frequency (%)	OR (95% CI)	P value*
RANTES -28 C/G		C/C	C/G	G/G	G			
Near-fatal asthma	48	26 (54.2%)	18 (37.5%)	4 (8.3%)	27.1	2.926 (1.414-6.055)	.006	
Mild-to-moderate asthma	134	108 (80.6%)	21 (15.7%)	5 (3.7%)	11.6	0.833 (0.446-1.554)	.677	
Atopic nonasthmatic	69	55 (79.7%)	14 (20.3%)	0 (0%)	10.1	0.880 (0.419-1.849)	.881	
Control	107	83 (77.6%)	23 (21.5%)	1 (0.9%)	11.7	1.0	—	
RANTES -403 G/A		G/G	G/A	A/A	A			
Near-fatal asthma	48	24 (50.0%)	18 (37.5%)	6 (12.5%)	31.3	1.277 (0.645-2.526)	.598	
Mild-to-moderate asthma	134	74 (55.2%)	47 (35.1%)	13 (9.7%)	27.2	1.035 (0.621-1.726)	.999	
Atopic nonasthmatic	69	42 (60.9%)	21 (30.4%)	6 (8.7%)	23.9	0.821 (0.443-1.520)	.821	
Control	107	60 (56.1%)	41 (38.3%)	6 (5.6%)	24.8	1.0	—	

OR, Odds ratio (reference group [controls] designated with an OR of 1.0).

*C/C versus C/G combined with G/G for -28 C/G, G/G versus G/A combined with A/A for -403 G/A.

TABLE II. Estimated haplotype frequencies for 4 groups

RANTES promoter site			Haplotype frequency (%)				P value*
Haplotype	-28	-403	Near-fatal asthma	Mild-to-moderate asthma	Atopic nonasthmatic	Control	
I	C	G	57.6	71.9	75.2	74.4	.010
II	C	A	15.3	16.5	14.7	14.1	.895
III	G	G	11.1	0.8	0.9	1.1	<.001
IV	G	A	15.9	10.7	9.3	10.5	.488

*The Pearson χ^2 test was used to determine whether significant differences were observed among 4 groups.**TABLE III.** Genotype and allele frequencies for RANTES -28 and -403 for Chinese children with asthma of different severities

Group	n	RANTES -28		P value*	RANTES -403		P value*
		C/C	C/G + G/G		G/G	G/A + A/A	
Near-fatal asthma	48	26 (54.2%)	22 (45.8%)	<.001	24 (50.0%)	24 (50%)	.119
Moderate asthma	50	35 (70.0%)	15 (30.0%)	.03	19 (38.0%)	31 (62.0%)	.004
Mild asthma	84	73 (86.9%)	11 (13.1%)	—	55 (65.5%)	29 (34.5%)	—

*Reference group: mild asthma.

mild asthma subgroups were 30% and 13.1%, respectively (OR, 2.84; $P = .03$; Table III). If we accept that near-fatal asthma delineates the severe end of the spectrum, the frequency of carriage of the -28G allele was parallel to the severity of asthma: lowest for the mild group (13.1%), intermediate for the moderate group (30%), and highest for the near-fatal analog (45.8%). The difference between near-fatal and mild asthma was also significant ($P < .001$). This result indicates that the RANTES -28G allele is specifically associated with relatively severe asthma. Similarly, the presence of the RANTES -403A allele was also significantly higher for the subjects with moderate asthma (62%) than for the subjects with mild asthma (34.5%, $P = .004$; Table III). However, the difference between the near-fatal and mild-asthma groups was not significant (50% and 34.5%, respectively; $P = .119$).

Association of RANTES genotypes with blood eosinophil counts, total serum IgE, specific IgE levels, and bronchial hyperresponsiveness

In the subset of 61 asthmatic children with available blood eosinophil counts, a statistically significant difference in mean eosinophil counts was observed between the moderate asthma and mild asthma subgroups (0.57 ± 0.31 vs $0.36 \pm 0.24 \times 10^6/\text{mL}$; $P = .007$). We therefore decided to investigate the impact of these 2 RANTES promoter polymorphisms on blood eosinophils for this subset of patients. Given the small number of children homozygous for the -28G allele, -28C/G and G/G genotypes were combined for the comparison; a significant difference between the RANTES -28 genotypes was demonstrated. The mean blood eosinophil count for asth-

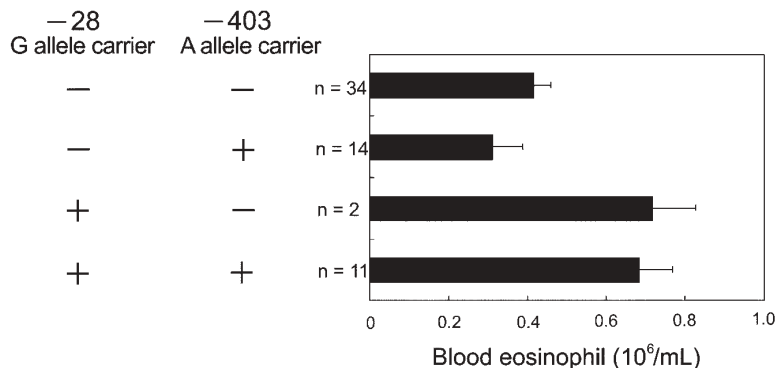


FIG 1. Mean blood eosinophil counts for asthmatic subjects with different genotypes at the RANTES -28 and -403 loci.

matic children carrying the RANTES -28G allele was significantly higher ($0.69 \pm 0.29 \times 10^6/\text{mL}$; $P = .002$) than the count for those homozygous for the -28C allele ($0.39 \pm 0.26 \times 10^6/\text{mL}$). In all 182 asthmatic children, when carriers of the -28G allele and those homozygous for the -28C variant were compared, no significant differences were demonstrated for the percentage of atopic subjects (83.3% vs 90.3%; $P = .302$) or total serum IgE concentrations (735.6 ± 677.4 vs 688.1 ± 575.2 kU/L; $P = .913$). The methacholine challenge tests were performed on 35 asthmatic children who had been symptom-free for at least 2 weeks. A positive bronchial hyperresponsiveness (BHR) value (FEV_1 had not decreased by 20% with a methacholine concentration of >8 mg/mL) was not observed for 3 of them, all of whom carried the -28C/C genotype. A significantly lower PC_{20} was demonstrated for carriers of the -28G allele compared with those homozygous for the -28C allele (0.60 ± 0.55 vs 2.71 ± 2.82 mg/mL; $P = .013$). In contrast to the RANTES -28C/G polymorphism, no significant effects were demonstrated from analysis to determine the associations between the RANTES -403 genotypes and blood eosinophil counts, atopy, total serum IgE levels, and BHR (data not shown).

With respect to the previously described allelic association between the -28G and -403A alleles,²⁰ it was also observed that most subjects bearing the -28G allele (91%) also carried the -403A allele. Blood eosinophil counts were further compared for asthmatic children who were classified according to 4 categories determined by genotypes at the RANTES -28 or -403 loci (Fig 1). The mean eosinophil count for carriers of both mutant alleles (-28G and -403A) was significantly higher relative to bearers of the -403A allele only (without the -28G mutation; $P = .009$) and those homozygous for the -28C and -403G alleles ($P = .017$). No significant difference was demonstrated when the latter 2 categories were compared. The mean eosinophil count for the 2 individuals who carried only the -28G allele (without -403A mutation) was comparable to that for carriers of both mutant alleles and higher than that for analogs with the -403A variant only (without -28G allele). Statistical signifi-

cance was not achieved, however, presumably because of the small sample size. These results provide further confirmation that the -28G allele, rather than the -403A variant, is responsible for the elevated circulating eosinophil counts.

DISCUSSION

This is the first study to investigate the potential role of polymorphisms of the CC chemokine system in the pathogenesis of life-threatening asthma. Although the mortality rate of asthma is low, for a small group of asthmatic patients severe, potentially fatal attacks remain a threat from early childhood. Thus, the identification of these at-risk individuals remains an important issue. To the best of our knowledge, this is the first study to demonstrate an association between polymorphisms within the RANTES promoter region and risk of near-fatal asthma attack.

The lack of association demonstrated between RANTES -28C/G polymorphism and mild-to-moderate asthma or atopy in this study is consistent with the results for Hungarian children¹⁷; however, 3 previously unknown findings were revealed. First, the frequency of the RANTES -28C/G polymorphism was significantly increased for children with near-fatal asthma compared with their counterparts who either had mild-to-moderate asthma or were nonasthmatic/nonatopic. The results of our haplotype analysis also support the association of near-fatal asthma with the -28C/G polymorphism. Second, the association of this polymorphism with moderate asthma (but not with mild asthma) suggests that the presence of the -28G allele (higher RANTES production) produces asthma of greater severity. Third, the -28G allele was also associated with increased blood eosinophil counts and a higher degree of BHR in our asthmatic children. We assume, however, that the lack of significance demonstrated in the Hungarian study for the association between this polymorphism and asthma severity might have been a reflection of reduced statistical power resulting from selection bias. Most patients in the Hungarian sample were classified as having mild-to-moderate asth-

ma; patients with severe persistent asthma were not included, and none of the cohort had experienced fatal or near-fatal attacks. This position is supported by Sandford et al,²⁴ who have shown that asthma severity significantly affects the results of genetic association studies for asthma. On the other hand, the discordance between our results and the results of Szalai et al¹⁷ might reflect the genetic heterogeneity among different ethnic populations. The prevalence of the RANTES -28G allele was 11.7% for our Chinese children—slightly, but not significantly, lower than for a Japanese population (16.6%; $P = .134$),¹⁴ but significantly higher than for reported Western samples (3.1%-4.4%; $P \leq .016$ in all cases).^{17,18,20,22} McDermott et al²⁰ simultaneously investigated the frequency of this allele for different populations. The -28G allele was almost never observed in Black and Hispanic individuals, was relatively uncommon in Caucasians (4%), and occurred most frequently in North American Asians (5.7%). In our cohort of Chinese children, the prevalence of the RANTES -403A allele was 24.8%, which is in good correlation with analogous results for a North American Asian population (27%).²⁰ It is significantly lower than for Japanese (37.6%-39.3%),^{14,21} and Black (35.7%-52.6%),^{15,20} but higher than for Caucasian ethnic groups (9.5%-20%).^{15-18,20,23} Comparing the results of these studies, it is interesting to note the higher frequency demonstrated for both the RANTES -28G and -403A alleles when the Asian populations were compared with the Western analogs. These findings suggest a genetic heterogeneity between Asian and Western populations with respect to regulation of RANTES expression, supporting the proposition that Asia is an area endemic for RANTES promoter polymorphisms. Thus, in terms of asthma severity, these polymorphisms might have a more substantial impact on Asian populations.

The mechanisms underlying the involvement of the RANTES gene in the pathogenesis of asthma of greater severity, including near-fatal attacks, remain to be identified and explained. RANTES has been strongly implicated in asthma, chiefly because of its potent effect on chemotaxis and activation of eosinophils.³⁰ The inflammation of the lungs in asthma is typically characterized by increased eosinophil influx.³¹ Associations between the clinical severity of asthma and the numbers of eosinophils in peripheral blood, bronchoalveolar lavage fluid, and respiratory epithelium have been demonstrated in a number of studies.^{31,32} Furthermore, airway eosinophils are further increased in individuals who have experienced fatal or near-fatal asthma attacks compared with patients with mild-to-moderate asthma and controls.^{32,33} In addition, in several studies involving asthmatic patients, blood eosinophil counts were correlated with the degree of BHR, possibly as a result of eosinophil-induced damage to the airway epithelium.³⁴ In the asthmatic children in our study, circulating eosinophil count was also associated with asthma severity, supporting the notion that eosinophils play an important role in asthma, as posited in previous studies. Eosinophils are the major effector cells in asthma, with the potential to cause tissue damage

through release of various cytotoxic mediators,¹³ enhance bronchial responsiveness, and cause airway obstruction.^{31,34} A strong relationship has been demonstrated between the number of eosinophils recruited to the lung and RANTES concentration.¹³

The C-to-G mutation at position -28 is adjacent to the NF- κ B binding site, which is a potent inducer of RANTES expression.³⁵ From functional analysis it has been demonstrated that the -28G allele elevates promoter activity and increases RANTES expression.¹⁴ We postulate that in asthmatic children carrying the RANTES -28G allele, chronically elevated levels of this CC chemokine might result in continuous recruitment and subsequent activation of a large number of eosinophils to the lung. When this elevation accompanies the release of toxic mediators, significant damage to the airway might result, necessitating airway remodeling and BHR. Therefore, this mutant variant of the RANTES gene might modulate asthma severity and further elevate RANTES levels during acute exacerbation, thus increasing the risk of fatal or near-fatal attacks.

In addition to targeting the eosinophil, it has been demonstrated that RANTES rapidly degranulates basophils and releases histamine,³⁶ selectively enhances B-cell IgE production,³⁷ and also participates in T-lymphocyte chemotaxis and activation.³⁸ These RANTES characteristics further support the proposed role in asthma pathogenesis. Furthermore, our findings are consistent with several genome-wide searches⁴⁻⁶ that have revealed linkages between chromosome 17q, containing the CC chemokine gene cluster (the RANTES gene lies on chromosome 17q11.2-q12) and asthma, eosinophil level, and BHR.

Given that atopy is a complex genetic trait, it seems unlikely that just 1 or 2 chemokines would prove sufficient for generating allergic disorders. This position might explain our inability to detect a significant effect for the RANTES -28C/G polymorphism on asthma or atopy susceptibility. Our view is that the presence of the RANTES -28G allele alone seems insufficient for generation of asthma or atopy; in asthmatic children, however, its influence might significantly perpetuate airway inflammation.

When the 4 groups of Chinese children in our study were compared, no significant difference was demonstrated for genotype or allele frequency for the RANTES -403G/A polymorphism. These results suggest that this polymorphism is not associated with mild-to-moderate asthma or atopy and that it is not a risk factor for near-fatal asthma attacks in Chinese children. Furthermore, this polymorphism does not significantly influence blood eosinophil level or degree of BHR. Unexpectedly, a higher frequency of -403A allele (higher RANTES production) was noted for the moderate-asthma subgroup compared with the mild-asthma analog, suggesting that the presence of this allele might be associated with asthma of greater severity. Despite the association between the RANTES -403 polymorphism and asthma severity demonstrated for the mild-to-moderate group, it is puz-

zling not to see such an association for near-fatal asthma, blood eosinophil count, or BHR. One possible explanation is that the association between the RANTES -403 genotype and the risk of near-fatal asthma is below our detection threshold. Surprisingly, we confirmed the strong linkage disequilibrium demonstrated between the -28G and -403A alleles²⁰; 91% of -28G-allele carriers in our cohort also bore the -403A allele, whereas only 9% did not. Because polymorphisms within a gene are often in linkage disequilibrium as a result of proximity, an observed effect for a polymorphism might actually be attributable to a nearby gene variant. It is therefore tempting to speculate that the effect of the RANTES -403A allele on the clinical severity of asthma might be a reflection of linkage disequilibrium with the nearby RANTES -28G allele. This explanation seems more likely given the fact that in this study comparable higher mean eosinophil levels were observed for carriers of both mutant alleles (-28G and -403A) and for those bearing the -28G allele but not the -403A mutation. Carriage of the -403A mutation alone did not affect blood eosinophils. This possibility has important implications for association studies of this gene, underscoring the necessity of accounting for linkage disequilibrium in the interpretation of results for these 2 polymorphisms. Furthermore, this possible confounding effect should be considered in any clinical investigations of the role of the RANTES promoter polymorphisms in inflammatory diseases. Combined genotyping and data analysis of these 2 polymorphisms might be required. Further study is necessary to explain the exact interaction of these 2 nearby polymorphisms.

Compared with previous studies assessing the role of the RANTES promoter polymorphisms in asthma, this investigation is unique in several respects. First, its predecessors primarily used Western populations, whereas our investigation focused on Chinese children whose mutant allele frequencies had not been reported. In addition, it seems that Asia might be an area endemic for the RANTES promoter polymorphisms. Further confirmation of this genetic heterogeneity is required because of the clinical consequences of this ethnic variation. Second, in previous investigations, the most severe cases were not enrolled, whereas our sample included 48 asthmatic children with histories of near-fatal attacks. Our observation illustrates the importance of selection of appropriate target patients for genetic studies of asthma. We support a research approach in which childhood near-fatal asthma is used as a suitable phenotype for the genetic dissection of asthma for 4 reasons. First, it is in general difficult to define the clinical phenotype for and severity of asthma. Many researchers believe that the traditional definition of asthma severity, which is based on clinical features before treatment, reflects the lack of control rather than the true severity.³⁹ Instead, asthma severity should be defined by minimum medication required to achieve control.³⁹ Nonetheless, this strategy remains somewhat arbitrary, because dose selection might vary within and between physicians. On the other hand, survivors of near-fatal attacks clearly delineate the severe

end of the spectrum, however. Second, though fatal and near-fatal attacks might have common causes, only rarely does asthma prove fatal. Richards et al⁴⁰ have confirmed the importance of a previous life-threatening attack as a marker for subsequent risk of death from asthma. Thus, the notion that study of near-fatal asthma attacks (as well as being of value in itself) might provide useful information with respect to the factors associated with fatal asthma attacks is an appealing one. Third, epidemiologic surveys have demonstrated different risk factors for childhood asthma compared with the adult variant.⁴¹ It might be rewarding, therefore, to include early-onset asthma patients, because the age of onset is, in part, a reflection of the genetic burden as reflected in disease risk.¹ Finally, the diagnosis of asthma is less difficult in children than in adults because it is less likely to be confounded by degenerative pulmonary diseases such as chronic obstructive pulmonary disease, chronic bronchitis, and emphysema.

In summary, we conclude that the RANTES -28C/G polymorphism exacerbates asthma severity and represents a genetic risk factor for life-threatening asthma attacks in Chinese children. The OR for the risk of asthma characterized by near-fatal incidents rather than the mild-to-moderate variant was 3.52 for children with the RANTES -28G allele versus those with the frequently occurring -28C/C genotype. In asthmatic children, the RANTES -28G allele was associated with an increased eosinophil level and a higher degree of BHR. If more analogous genetic markers are identified, assessment of the risk for life-threatening episodes of asthma by genotyping of susceptible children in early childhood might become feasible. In addition, it seems reasonable to suggest that the strong linkage disequilibrium between the -28G and -403A alleles is a potential confounder that must be considered in the design and interpretation of RANTES-gene association studies.

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